Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 813 (2004) 247–254
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Direct injection, column switching–liquid chromatographic technique for the estimation of rabeprazole in bioequivalence study

Sonu Sundd Singh*, Manish Jain, Hiten Shah, Sapna Gupta, Purav Thakker, Ruchy Shah, Braj Bhushan Lohray

Zydus Research Centre, Sarkhej-Bavla N.H. No. 8A, Moraiya, Ahmedabad 382213, India

Received 27 April 2004; accepted 23 September 2004

Abstract

A rapid, simple and sensitive high-performance liquid chromatography–ultra violet (HPLC–UV) method with column switching between sample pre-treatment column and analytical column was developed for the quantitation of rabeprazole in human plasma; on a Bio-Sample Analysis system (Co-sense® for BA) from Shimadzu Corporation, Kyoto, Japan. Zaleplon was used as an internal standard. The method was validated as per USFDA guidelines for the concentration range of 20.0–1200.0 ng/mL and the correlation coefficient were found to be better than 0.999. Recovery of rabeprazole as well as the internal standard from human plasma was more than 90.0%. Rabeprazole was stable in human plasma for 4 months at $-70 \pm 5\,^{\circ}C$ and for 20.0 h at ambient temperature. In the auto sampler, the drug was stable for 24.0 h at 4 °C. The method was specific as there were no interfering peaks in the human plasma eluting at the retention times of the rabeprazole and the internal standard. The frozen plasma samples containing rabeprazole were stable to three freeze thaw cycles. The bioanalytical method was rugged in terms of inter- and intra-day accuracy and precision. The method was simple, specific, sensitive, precise, accurate and suitable for bioequivalence and pharmacokinetic studies. It was successfully applied to the pilot bioequivalence study of 20 mg rabeprazole tablet of German Remedies Ltd. (A division of Cadila Healthcare Ltd.), India versus Pariet tablet of Eisai Ltd. & Janssen-Cilag Ltd., Japan in male human subjects.

Keywords: Rabeprazole; Human plasma; Validation and bioequivalence

1. Introduction

Rabeprazole is a potent gastric proton pump inhibitor, which causes dose dependent acid secretion [1,2]. It has faster onset of action and lower potential of drug interaction as compared to omeprazole. Rabeprazole is indicated in the treatment of erosive or ulcerative gastro oesophageal reflux disease (GERD), healing of duodenal ulcer and treatment of pathological hypersecretory conditions, including Zollinger Ellison syndrome.

The single dose oral pharmacokinetics of rabeprazole has been reported [1] to be linear in the range of 10.0–80.0 mg. The reported time point of maximum plasma concentration (T_{max}) was 2.9–3.8 h and was independent of dose. The pharmacokinetics of rabeprazole was generally similar after single and multiple daily dose of 20.0 and 40.0 mg. The plasma protein binding was around 96.3%. Rabeprazole was rapidly cleared by hepatic metabolism and renal excretion. The elimination half-life of rabeprazole was approximately 1.0 h and oral clearance ranged from 0.26 to 0.5 L/h/kg which was independent of dose [1]. Rabeprazole is metabolized by human Cytochrome P450 enzymes CYP2C19 and CYP3A4 to desmethyl and sulfone metabolites. The major metabolite is thio-ether carboxylic acid compound [1].

Correct interpretation of the pharmacokinetic data obtained from a bioequivalence study can only be made if the bio-analytical methodology employed is adequately sensitive, accurate and precise. The sensitivity and accuracy of a bio-analysis is governed primarily by sample pretreatment and chromatographic conditions. The various tech-

* Corresponding author. Tel.: +91 2717 250 801–805; fax: +91 2717 250606.
E-mail address: sonusingh@zyduscadila.com (S.S. Singh).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.09.053

niques conventionally employed for sample pre-treatment are liquid–liquid extraction (LLE), protein precipitation, solid phase extraction (SPE). In the past [3–8], rabeprazole has been analyzed in biological matrices using similar techniques.

The technique of injection of the plasma sample directly into liquid chromatograph without any sample processing has become quite popular in the present age of high-throughput. There are a couple of earlier reports [9,10] on the quantitation of rabeprazole in rat and dog plasma by similar technique. This technique of on-line sample clean up provides the advantages of high reproducibility, reduced labor of analysis, rapid de-proteinization of sample and over all cost effectiveness over the conventional techniques.

In the present study, direct plasma injection followed by column switching between the pre-treatment column and the analytical column has been employed to achieve the desired speed and reproducibility on the integrated system named Co-sense® for BA Bio-analysis system from Shimadzu, Japan. In this procedure, plasma is directly led to the pre-treatment column where the drug component gets concentrated and trapped, while it is being de-proteinised. The drug components trapped on the pre-treatment column are subsequently introduced into the analytical column by simple switching of the valve.

The outer surface of stationary phase (silica gel) of a sample pre-treatment column "Shim-Pack MAYI-ODS" is coated with a water-soluble polymer and only the pore interior is chemically modified with octadecyl group. Macromolecules such as proteins cannot penetrate the pore interior blocked by water-soluble polymer on the outer surface, and thereby they are easily eluted out. The low molecular weight analytes are able to permeate into the pore interior and are retained by the stationary phase of inner surface.

The present method involves minimal sample preparation as compared to high-performance liquid chromatography (HPLC) [3–7] or liquid chromatography–mass spectroscopy (LC–MS) [8] techniques. This property renders it cost effective as the sample processing time is saved, lesser manpower is required, expenses on extraction solvents and costly solid phase extraction cartridges are curtailed. Since the several steps involved in sample preparation are avoided, the chances of introduction of processing errors are reduced to a large extent. The obvious disadvantages are longer run times as compared to LC–MS methods where run times are as short as 3–4 min and moreover, the present method is not enantioselective.

A detailed pre-study method validation was conducted as per USFDA guidelines [11] to establish the linearity, specificity, accuracy, precision and stability (bench top, autosampler, freeze thaw, long term and stock solution) of the method. The method was applied to the quantitation of rabeprazole in a single dose, crossover bioequivalence study of 20 mg rabeprazole tablets of German Remedies Ltd. (A division of Cadila Healthcare Ltd.), India versus Pariet tablet containing 20 mg rabeprazole of Eisai Ltd. & Janssen-Cilag Ltd., Japan, in healthy, adult, male, human subjects. The study was carried out with the objective of obtaining marketing authorization for rabeprazole tablets in the Indian market.

2. Experimental

2.1. Reagents and chemicals

Rabeprazole sodium (purity 99.87%) and zaleplon (purity 99.23%) working standards were provided by Cadila Healthcare Ltd. Acetonitrile Omnisolv® was purchased from Merck, KGaA, Darmstadt, Germany. Ammonium acetate (GR Grade) was obtained from Merck, India. Ammonia (GR Grade) was from Qualigens fine chemicals, India. Blank Plasma was purchased from Gujarat Blood Bank, Pathology Laboratory & R.I.A. Unit, Ahmedabad, India. HPLC Type II Water from Millipore's Milli-Q System was used throughout the pre-study validation and analysis.

2.2. Instrumentation

The liquid chromatograph consisted of an integrated Bio-Sample Analysis system, Co-sense® for BA, and a SIL-HT*c* auto sampler from Shimadzu Corporation, Kyoto, Japan. The de-proteinization and pre-concentration of samples was performed on "Shim-Pack MAYI ODS" (10 mm × 4.6 mm) pre-treatment column from Shimadzu Corporation, Kyoto, Japan. Subsequent separations were carried out on Water's Symmetry C18, (250 mm × 4.6 mm; 5.0 μ) analytical column. Rabeprazole and the internal standard was detected using a UV detector (SPD-10AVP, Shimadzu) operated at 290 nm. Class VP software 6.12 Version, Shimadzu Corporation, Kyoto, Japan was used to acquire and process all chromatographic data.

2.3. Preparation of stock and working solutions

The stock solution (0.1 mg/mL) of rabeprazole (Fig. 1) was prepared by dissolving appropriate amount of rabeprazole sodium working standard in 0.1% (v/v) aqueous solution of diethyl amine. The stock solution was further serially diluted in 0.1% (v/v) aqueous solution of diethyl amine to obtain working solutions of calibration standards (24,000.0, 18,000.0, 12,000.0, 6000.0, 4000.0, 2000.0, 800.0 and 400.0 ng/mL) and quality control (QC) samples (20,000.0, 10,000.0 and 1000.0 ng/mL) of the rabeprazole.

A 1.0 mg/mL stock solution of zaleplon (internal standard, Fig. 1) was prepared in water:acetonitrile (50:50, v/v) and subsequently diluted with the same diluent to obtain a 1250.0 ng/mL working internal standard solution.

2.4. Preparation of calibration standards and quality control sample

To 950.0 μL of the drug free plasma, 50.0 μL of working solutions of rabeprazole was added to yield final respective

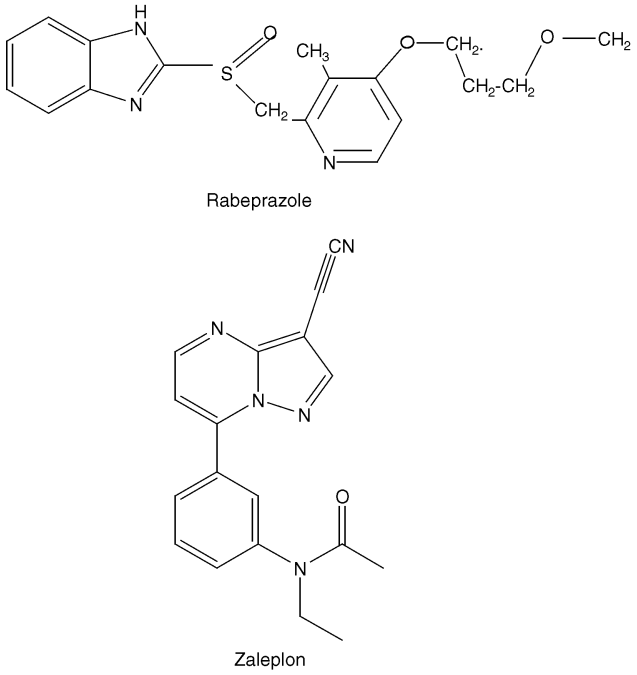

Fig. 1. Structures of rabeprazole and internal standard.

concentrations as 1200.0, 900.0, 600.0, 300.0, 200.0, 100.0, 40.0 and 20.0 ng/mL of rabeprazole in plasma. To each calibration standard, 50.0 μL of internal standard working solution and 10 μL of diethyl amine was added and vortexed. QC samples (1000.0, 500.0 and 50.0) were prepared in same manner.

2.5. Sample preparation

To 1000.0 μL of subject sample, 50.0 μL of working solution of internal standard was added and vortexed for 45 s. The vortexed samples were centrifuged for 10 min at 10,000.0 rpm and the supernatant was transferred to 1.0 mL HPLC vial for direct injection into HPLC.

2.6. Chromatographic conditions

The analytical mobile phase consisting of 25.0 mM ammonium acetate buffer (pH adjusted to 7.0 with dilute ammonia solution) and acetonitrile in the ratio 70:30 (v/v) was continuously passed through the analytical column with flow rate of 1.0 mL/min. At the same time, the pre-treatment mobile phase premix of 10 mM ammonium acetate buffer (pH adjusted to 7.0 with dilute ammonia solution) and acetonitrile (95:5, v/v) was continuously passed through the pre-treatment column at a flow rate of 0.25 mL/min.

About 300.0 μL of human plasma samples maintained at 4 °C in autosampler was directly injected into the pre-treatment column. The sample de-proteinization and pre-concentration (Fig. 2a) was carried out within 4 min by pre-treatment mobile phase at a total flow rate of 2.0 mL/min. Thereafter, the column switch valve was switched so that

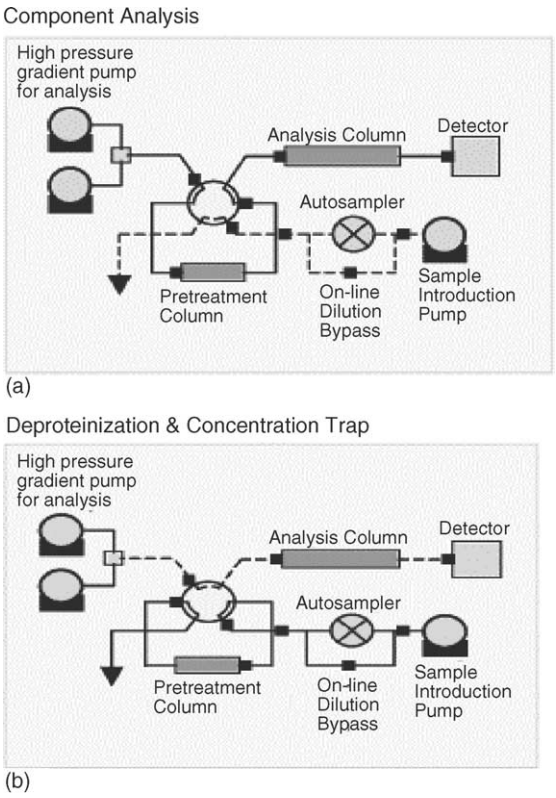

Fig. 2. (a) Flow diagram of drug analysis procedure. (b) Flow diagram of de-proteinization and concentration trap.

the analytical column was in line with the pre-treatment column and the detector (Fig. 2b) with the analytical mobile phase flowing at the rate of 1.0 mL/min. Simultaneously, the pre-treatment column was also being washed with pre-treatment mobile phase at a flow rate 2.0 mL/min for 4.0 min, followed by equilibration for next injection with the flow rate of 0.25 mL/min. The chromatographic separation is demonstrated in Fig. 3.

2.7. Bio-analytical method validation

2.7.1. Linearity

To establish the linearity, a series of eight calibration standards (1200.0, 900.0, 600.0, 300.0, 200.0, 100.0, 40.0 and 20.0 ng/mL) were prepared by adding 50.0 μL of respective working solutions of rabeprazole and internal standard to 950.0 μL of drug free human plasma and analyzed in five-fold. A correlation of more than 0.99 was desirable and *F*-test for lack of fit was applied to all the calibration curves The lowest standard on the calibration curve was to be accepted [11] as the lower limit of quantitation (LLOQ) if the analyte response in the standard was five times more than that of drug free (blank) plasma. In addition, the analyte peak in LLOQ sample should be identifiable, discrete, and reproducible with a precision of 20.0% and accuracy within 80.0–120.0%. The deviation of standards other than LLOQ from the nominal concentration should not be more than ±15.0%. It was de-

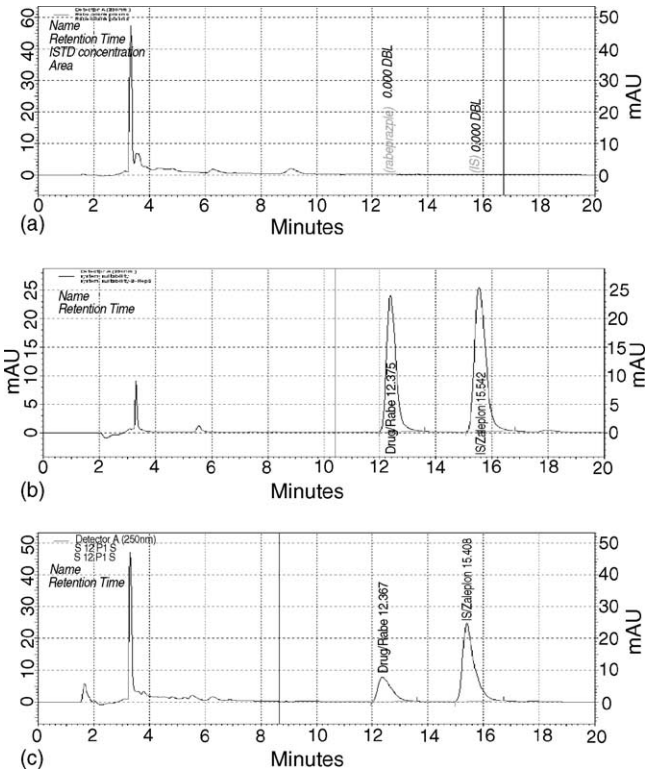

Fig. 3. Representative chromatogram in human plasma: (a) blank plasma; (b) plain standard (rabeprazole concentration 500 ng/mL and internal standard 62.5 ng/mL); and (c) plasma sample of a subject (rabeprazole concentration 241 ng/mL and internal standard 62.5 ng/mL).

sirable that a minimum of six non-zero standards, including LLOQ, met the above criteria.

2.7.2. Specificity

At least six randomly selected drug free human plasma samples were directly injected into HPLC to determine the extent to which endogenous plasma components may contribute to the interference at retention times of analyte and internal standard. The specificity of method was further tested by running plasma samples spiked with over the counter (OTC) drugs namely; paracetamol, ibuprofen, ampicillin, diclofenac sodium, nicotinamide and amoxycillin at the concentration of 50.0 ng/mL to check for their interference at the retention time of rabeprazole and internal standard. The peak response for OTC drugs were compared with that for plasma samples containing 20.0 ng/mL rabeprazole and 62.5 ng/mL of internal standard.

2.7.3. Recovery (extraction efficiency) from plasma

Recovery of rabeprazole was evaluated by comparing the mean peak responses of six quality control samples of low, medium and high concentrations to mean peak responses of six plain standards of equivalent concentration. Similarly, the recovery of Internal standard was evaluated by comparing the mean peak responses of six quality control samples to mean peak responses of six plain standards of similar concentration. As per the acceptance criteria [11], the recovery of the analyte need not be 100.0%, but the extent of recovery of an analyte should be consistent, precise and reproducible.

2.7.4. Accuracy and precision

Intra-day accuracy and precision were evaluated by replicate analysis of rabeprazole at different concentrations in human plasma. The run consisted of a calibration curve plus six replicates each of lower limit of quantification, low, medium and high quality control samples. The inter-day accuracy and precision were also assessed by analysis of LLOQ, low, medium and high quality control samples for rabeprazole on five separate occasions. The precision of the method was determined by calculating the percent coefficient of variation (% CV) for the concentrations obtained for different determinations. For the evaluation of precision, the deviation of each concentration level from the nominal concentration was expected to be within ±15.0% except for the LLOQ, for which it should not be more than 20.0% [11]. Similarly, the mean accuracy should not deviate by ±15.0% of the nominal concentration except for the LLOQ where it should not deviate by more than ±20.0% of the nominal concentration.

2.7.5. Stability

In order to determine the long-term stability of rabeprazole in human plasma, six aliquots of each, low and high QC samples were kept in deep freezer at −70 ±5 °C for 4 months. The samples were processed along with precision and accuracy batch prepared from stored working solutions and the concentrations obtained were compared with theoretical value of QC samples.

Six aliquots each of the low and high QC samples were kept at ambient temperature (20–30 °C) for 20.0 h in order to establish the short-term stability of rabeprazole in human plasma. Thereafter, 50 μL of internal standard was added and the samples were analyzed. The concentrations thus obtained were compared with the nominal value.

In order to establish the auto-sampler stability of rabeprazole in human plasma matrix, six aliquots each of low and high QC samples were stored at 4 °C in auto-sampler for 24.0 h. Thereafter, samples were analyzed and concentrations compared with the nominal value. The solution stability of rabeprazole was determined by storing working solutions of 500.0 ng/mL at 2–8 °C for 40 days. After 40 days, the mean areas of rabeprazole from four replicate chromatographic runs were compared to that of mean area of freshly prepared solutions of same concentration.

Effect of three freeze and thaw cycles on stability of plasma samples containing rabeprazole was determined by subjecting six aliquots each of low and high quality control samples (previously frozen at −70 ± 5 °C) to three freeze thaw cycles. After the completion of third cycle, the samples were analyzed and the experimental concentrations were compared with the nominal values. All stability samples qualified the test if the deviation from the nominal value was within ±15.0%.

2.8. Bioequivalence study design

Open label, balanced, randomized, two-treatment, two-sequence, two-period, single dose, crossover bioequivalence study of rabeprazole tablet containing 20 mg rabeprazole of German Remedies Ltd. (A division of Cadila Healthcare Ltd.), India, with that of Pariet tablet containing 20 mg rabeprazole of Eisai Ltd. & Janssen-Cilag Ltd., Japan, in healthy, adult, male, human subjects under fasting conditions was conducted in accordance with the current good clinical practice (GCP) and FDA guidelines [12].

Study was performed on healthy, willing, 13 male volunteers 18–45 years of age, after they had been informed of the purpose, protocol and risk involved in the study. All subjects gave written informed consent and the protocol was approved by local ethics committee. Subjects were screened 21 days prior to administration of drug. All subjects were instructed to abstain from any xanthine containing food and beverages (like chocolates, tea, coffee or cola drinks), cigarettes, tobacco and alcoholic products for 48 h prior to dose administration and throughout their stay at the clinical facility. All subjects were in fasting status for at least 10 h before dosing. They were not allowed to drink water from 1 h pre-dose to 2 h post-dose and thereafter, free access to drinking water was allowed.

A total of 16 blood samples were collected during each period. The venous blood samples 6 mL including (1 mL discarded heparinised blood) were withdrawn via an indwelling cannula at pre-dose and at 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, 12, 18, and 24 h following drug administration in each period of the study. The samples were collected in pre-labeled vacutainers containing EDTA as the anti-coagulant and centrifuged at 3000 rpm for 15 min at 15 °C and plasma was collected in pre-labeled sample collection tube. Since rabeprazole is very acid labile [2], and therefore 35.0 μL of diethyl amine was added to 2.5 mL of plasma and vortexed to stabilize it in plasma. A wash out period of 7 days was observed between the two phases of the study. The samples were stored in the deep freezer at -70 ± 5 °C until analyzed by a validated HPLC method.

2.9. Pharmacokinetic and statistical analysis

The pharmacokinetic parameters namely: maximum plasma concentration (C_{max}), time point of maximum plasma concentration (T_{max}), area under the plasma concentration–time curve from 0 h to the last measurable concentration (AUC_{0-t}), area under the plasma concentration−time curve from 0 h to infinity ($AUC_{0-\infty}$), elimination rate constant (λ_Z) and half-life of drug elimination during the terminal phase ($t_{1/2}$) were calculated using WinNonlin Professional Software- Version 4.0.1.

Statistical analysis of pharmacokinetic parameters was carried out using SAS® Release 8.2 (SAS Institute Inc., USA) for un-transformed and ln-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$. Based on the statistical results of 90.0% confidence intervals for the ratios of the means of ln-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ conclusion was drawn as to whether the test product was bioequivalent to the reference product. Bioequivalence was to be concluded if the 90.0% confidence interval fell within the bioequivalence range 80.0–125.0% [13] for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$. In-process and retrospective audits of the study were conducted by Quality Assurance Department of Zydus Research Centre.

3. Results and discussion

3.1. Method optimization

Initially, the method development, was started on HPLC (model 2010, Shimadzu) equipped with an UV detector. After taking various trials with different columns such as Inertsil ODS-3-V (GL Sciences), YMC-ODS, Luna C18 and several different mobile phase compositions the chromatography was finalised on water's symmetry C18 column and mobile phase of 25.0 mM ammonium acetate pH 7.0 and acetonitrile (70:30, v/v). Zaleplon was finally selected as an internal standard after trying a number of compounds such as, itopride, etoricoxib, ornidazole, atorvastatin, omeprazole, etc. Different extraction methods such as precipitation, solid phase extraction and liquid–liquid extraction were tried to obtain maximum recovery and minimum or no interference of plasma. The maximum recovery that could be obtained by these conventional techniques was merely 60.0%.

Therefore, it was a conscious decision to shift the method development on Bio-Sample Analysis system, which is an integrated HPLC system with column switching technique. The same chromatographic conditions as above were applied for separation of rabeprazole and internal standard. Attempts were made for the de-protinization of plasma samples on "Shim-Pack MAYI-ODS" pre-treatment column using different pre-treatment mobile phases with different concentration of organic solvent for different duration of pre-treatment time to obtain maximum recovery and no interference of plasma. The major draw back was the deterioration of the analyte peak shape after a few injections of plasma with a very high backpressure of column due to blockage of pre-treatment column with large molecules of plasma. The problem was eliminated by centrifuging the plasma samples at 10,000.0 rpm for 10 min and installing a titanium frit of 0.2 μ after the six port column-switching valve and before the pre-treatment column. Since the method involved direct introduction of plasma sample into liquid chromatograph, and there was no option to increase the overall sensitivity of the method by offline pre-concentration of the analyte and reconstitution in lower volumes of solvent. Therefore, a high injection volume of 300.0 μL had to be used to obtain the desired sensitivity.

Table 1
Summary of rabeprazole calibration standards in human plasma.

Concentration added (ng/mL)	Mean concentration found (ng/mL)	S.D.	CV (%)	Accuracy (%)	*n*
20	20.57	1.8	8.70	102.85	5
40	38.60	1.1	2.81	96.50	5
100	97.95	1.5	1.53	97.95	5
200	189.06	1.0	0.53	94.53	5
300	300.85	2.0	0.67	100.28	5
600	608.58	8.7	1.43	101.43	5
900	897.09	3.3	0.37	99.68	5
1200	1228.52	13.5	1.10	102.38	5

Table 2
Summary of rabeprazole calibration curve parameters in human plasma

Calibration curve	*Y* intercept	Slope	Correlation (*r*)	*F*-test for lack of linearity	
				F	*p*-value
1	0.0	1215.6	0.999	12521.8	<0.01
2	0.0	1211.3	0.999	10858.9	<0.01
3	4.0	1544.4	1.000	42310.9	<0.01
4	2.3	1504.6	0.999	10759.6	<0.01
5	3.2	1651.1	1.000	22097.2	<0.01
Mean	1.9	1225.4	0.999	–	–
S.D.	1.9	357.0	0.000	–	–

3.2. Bio-analytical method validation

3.2.1. Linearity and lower limit of quantitation

Ratio of detector response for rabeprazole to internal standard was used for linear regression analysis of each calibration curves. Back calculations were made from the calibration curves to determine rabeprazole concentrations of each calibration standard. Calibration curves were found to be linear over the range of 20.0–1200.0 ng/ml, with the co-efficient of correlation better than 0.999 (Table 2). *F*-test on lack of fit at 99% confidence level was performed and it exhibited non-significance (Table 2). The lower limit of quantitation of was 20.0 ng/ml, with a coefficient of variation of 8.7% and accuracy of 102.85% (Table 1) which complied the acceptance criteria.

3.2.2. Specificity

No significant interfering peaks were observed at the retention times of either analyte or internal standard in six different lots of drug free human plasma samples used for analysis.

Similarly, the chromatography was devoid of any interference at the retention times of either analyte or internal standard, when tested by running plasma samples spiked with over the counter medicines, like aspirin, ibuprofen, ampicillin, diclofenac sodium and xanthine compounds like caffeine and nicotine which could be the potential interference's in the assay.

3.2.3. Recovery

The recovery of rabeprazole from human plasma was significantly high. The mean recovery values for rabeprazole in human plasma were 92.99–95.63% with coefficient of variation of 0.61–4.6% as presented in Table 3. The mean recovery for internal standard was 93.56%.

3.2.4. Accuracy and precision

The intra-day accuracy ranged between 99.09 and 108.15% with coefficient of variation of 0.79–3.24% (Table 4). The observed inter-day accuracy ranged between 97.34 and 100.37% (Table 5) and the coefficients of variation was between 2.88 and 7.85%. The data provides sufficient evidence for the accuracy and ruggedness of method.

3.2.5. Stability

Rabeprazole was stable at $-70 \pm 5\,^{\circ}C$ for 4 months (long-term stability) in human plasma. The percent changes (bias) for rabeprazole concentration over the stability-testing

Table 3
Recovery (extraction efficiency) of rabeprazole in human plasma

QC samples	Concentration (ng/mL)	Recovery found (%)	C.V. (%)	*n*
Low	50	95.63	4.60	6
Mid	500	92.99	0.62	6
High	1000	94.59	0.61	6

Table 4
Intra-day accuracy of rabeprazole in human plasma

QC samples	Concentration added (ng/mL)	Mean Concentration found (ng/mL)	S.D.	CV (%)	Accuracy (%)	*n*
LLOQ	20	21.63	0.7	3.24	108.15	6
Low	50	51.23	1.08	2.11	102.45	6
Mid	500	507.07	4.00	0.79	101.41	6
High	1000	990.95	12.93	1.30	99.09	6

Table 5
Inter-day accuracy of rabeprazole in human plasma

QC samples	Concentration added (ng/mL)	Mean concentration found (ng/mL)	S.D.	CV (%)	Accuracy (%)	*n*
LLOQ	20	19.55	1.53	7.85	97.77	30
Low	50	48.98	2.46	5.01	97.97	30
Mid	500	501.86	14.47	2.88	100.37	30
High	1000	973.38	58.02	5.96	97.34	30

Table 6
Summary of stability of rabeprazole in human plasma

Stability	Concentration added (ng/mL)	Mean concentration found (ng/mL)	S.D.	CV (%)	Bias (%)	*n*
Long term (4 months)	50	46.05	2.78	6.04	−7.90	6
	1000	925.51	61.94	6.96	−7.45	5
Short term 20 h	50	53.82	0.65	1.20	7.64	5
	1000	1080.53	26.07	2.41	8.05	6
Auto sampler (24 h)	50	49.76	1.13	2.26	−0.48	6
	1000	988.10	11.08	1.12	−1.19	6
Freeze	50	44.34	1.03	2.32	−11.32	6
Thaw	1000	986.78	16.11	1.63	−1.32	6

period of 4 months in deep freezer at $-70 \pm 5\,^{\circ}C$ were −7.90 and −7.45% at the concentrations of 50.0 ng/mL and 1000.0 ng/ml, respectively. Results are presented in Table 6.

Rabeprazole was found to be stable for 20.0 h in human plasma at ambient temperature (20–30 °C). The percent bias observed were 7.64 and 8.05% at the concentration of 50.0 and 1000 ng/mL (i.e. LQC and HQC), respectively (Table 6). In the auto-sampler maintained at 4 °C, the plasma samples of rabeprazole were stable for 24.0 h and the percent bias for the drug concentrations after 24.0 h were −0.48 and −1.19% at LQC and HQC levels (Table 6). LQC and HQC samples of, rabeprazole were found to be stable even after subjecting to three freeze thaw cycles. The percent bias observed after three freeze thaw cycles were −11.32 and −1.32% at the two concentrations as shown in Table 6.

Working solutions of rabeprazole and internal standard were found to be stable for 40 days at 2–8 °C.

3.3. Statistical evaluation of pharmacokinetic parameters

The pharmacokinetics of the two formulations was compared in terms of extent (AUC_{0-t} and $AUC_{0-\infty}$) and rate (C_{max} and T_{max}) of absorption. The mean pharmacokinetic parameters for the test and reference formulation are presented in Table 7.

Table 7
Pharmacokinetic parameters of rabeprazole after the administration of an oral dose of 20 mg of test and reference formulations ($N = 13$)

Pharmacokinetic parameters	Reference formulation (*R*) (mean ± S.D.)	Test formulation (*T*) (mean ± S.D.)
T_{max} (h)	4.23 ± 0.95	4.35 ± 0.20
C_{max} (ng/mL)	491.55 ± 224.58	451.80 ± 229.38
AUC_{0-t} (ng h/mL)	880.07 ± 442.47	771.80 ± 433.63
$AUC_{0-\infty}$ (ng h/mL)	925.78 ± 453.26	898.54 ± 395.03
$T_{1/2}$ (h)	0.99 ± 0.42	1.91 ± 3.56
λ_Z (1/h)	0.80 ± 0.29	0.79 ± 0.36

3.3.1. Rate of absorption

The mean C_{max} for the reference and test formulation were 491.55 ± 224.58 and 451.80 ± 229.38, respectively (Table 8). The two one-sided 90% confidence interval for the ratio of the ln-transformed means of C_{max} was found to be 91.39–111.25% (Table 8) was in accordance with the acceptance limit of 80.0–125.0%, required for the conclusion of

Table 8
Summary of statistics of pharmacokinetic parameters of rabeprazole

Parameters	90% confidence limit	Limit (%)
AUC_{0-t} (ng h/mL)	85.54–104.87	
$AUC_{0-\infty}$ (ng h/mL)	86.74–103.81	80–125
C_{max} (ng/mL)	91.39–111.25	

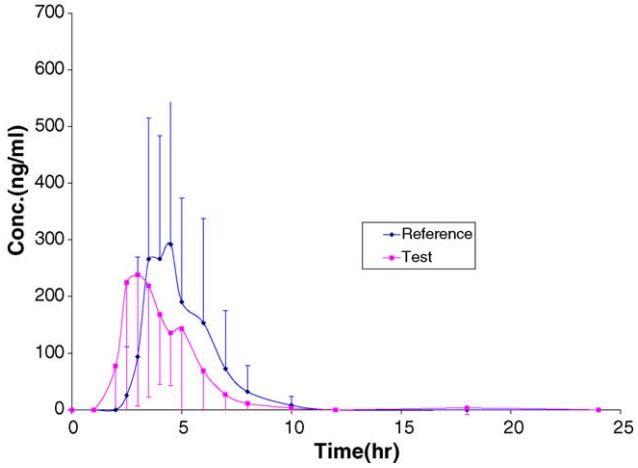

Fig. 4. Mean plasma concentrations vs. time graph of rabeprazole after administration of test and reference formulations to healthy, adult, male and human subjects under fasting condition.

bioequivalence. The mean T_{max} for reference and test formulations were 4.23 ± 0.95 and 4.35 ± 0.20 h (Table 7), respectively.

3.3.2. *Extent of absorption*

The mean AUC_{0-t} and $AUC_{0-\infty}$ for the reference and test formulation are presented in Table 7. The two one-sided 90.0% confidence interval for the ratios of the ln-transformed means of AUC_{0-t} and $AUC_{0-\infty}$ were found to be 85.54–104.87% and 86.74–103.81% (Table 8), respectively. Both the intervals complied the acceptance limit of 80.0–125.0%, essential for the two formulations to be bioequivalent.

These observations confirm that the test formulation, 20 mg rabeprazole tablet of German Remedies Ltd. (A division of Cadila Healthcare Ltd.), India, was bioequivalent to the reference product Pariet tablet containing 20 mg rabeprazole of Eisai Ltd. & Janssen-Cilag Ltd., Japan in terms of rate and extent of absorption. The mean concentration versus time graphs for the two formulations are shown in Fig. 4. Moreover, there were no reports of any adverse events during the conduct of the study.

4. Conclusions

The high-throughput bio-analytical methodology described in this manuscript was simple, specific, sensitive accurate and precise. The method involved direct plasma injection in liquid chromatograph with UV detection, and column switching, which resulted in on-line sample purification, pre-concentration and separation using isocratic chromatographic conditions. The method allowed the estimation of rabeprazole up to 20.0 ng/mL in human plasma with high degree of accuracy and precision. The method was successfully applied to bio-analysis of bioequivalence study in human subjects. The statistical analysis of pharmacokinetic parameters obtained for the two formulations confirmed that the test and reference formulation administered in the study were bioequivalent in terms of rate and extent of absorption.

Acknowledgements

We are thankful to Dr. Vidya Bhushan Lohray (Sr. vice president) for the constant support and encouragement and also to Dr. Shri Prakash Singh and Mr. Kinjal Joshi from Quality Assurance for auditing the study.

References

[1] H.D. Langtry, A. Markham, Drugs 58 (4) (1999) 725.
[2] Pariet (Rabeprazole, 10 mg and 20 mg) electronic package insert, Jannsen-Cilag™ Eisai Co. Ltd., Tokyo, Japan.
[3] S. Yasuda, A. Ohnishi, T. Ogawa, Y. Tomono, J. Hasegawa, H. Nakai, Y. Shimamura, N. Morishita, Int. J Pharmacol. Ther. 32 (9) (1994) 466.
[4] S. Takakuwa, S. Chiku, H. Nakata, T. Yuzuriha, N. Mano, N. Asakawa, J. Chromatogr. B 673 (1) (1995) 113.
[5] W.F. Keane, S.K. Swan, I. Grimes, T.J. Humphries, J. Clin. Pharmacol. 39 (9) (1999) 927.
[6] S. Yasuda, S. Higashi, M. Murukami, Y. Tomono, M. Kawaguchi, Int. J. Pharmacol. Ther. 37 (5) (1999) 249.
[7] H. Nakai, Y. Shimamura, T. Kanazawa, S. Yasuda, M. Kayano, J. Chromatogr. B 660 (1) (1994) 211.
[8] Y. Zhang, X. Chen, Q. Gu, D. Zhong, Anal. Chim. Acta 523 (2) (2004) 171.
[9] S.H. Shim, S.J. Bok, K.I. Kwon, Arch. Pharm. Res. 17 (6) (1994) 458.
[10] N. Mano, Y. Oda, S. Takakuwa, S. Chiku, H. Nakata, N. Asakawa, J. Pharm. Sci. 85 (9) (1996) 903.
[11] Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration Centre for Drug Evaluation and Research (CDER), Centre for veternary Medicine (CVM), May 2001 BP, website: http://www.fda.gov/cder/guidance/index.htm.
[12] FDA Guidance for Industry, Biaoavaliability Studies for Orally Administered Drug: Products-General Considerations, US Department of Health and Human Services, Food and Drug Administration, Centre for Drug Evaluation and Research (CDER), 2000, website: http://www.fda.gov/cder/guidance/index.htm.
[13] FDA Guidance for Industry, Statistical Approaches to Establishing Bioequivalence, US Department of Health and Human Services, Food and Drug Administration, Centre for Drug Evaluation and Research (CDER), 2001, website: http://www.fda.gov/cder/guidance/index.htm.